ORIGINAL ARTICLE

Environmental risk factors for type 1 diabetes in Rome and province

N Visalli, L Sebastiani, E Adorisio, A Conte, A L De Cicco, R D'Elia, S Manfrini, P Pozzilli, the IMDIAB Group

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See end of article for authors' affiliations

Correspondence to: Prof. P Pozzilli, Università Campus Biomedico, Via Longoni 83, 00155 Rome, Italy; p.pozzilli@unicampus.it

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Background: In subjects genetically susceptible to type 1 diabetes, exposure to environmental factors during the gestational period, the neonatal period, and the first years of life is thought to play an important role in triggering the immune process leading to β cell destruction.
Aims: To investigate risk factors for inhabitants of continental Italy.
Methods: A case-control study of 150 type 1 diabetes cases and 750 control subjects (age range 6–18 years) was carried out in Rome and its province, measuring the exposure to environmental risk factors.
Results: Three environmental factors were found to occur significantly more in the diabetic group than in the controls. During the mothers' pregnancies, the one risk factor which proved to be higher in diabetics than in controls was maternal infectious disease. During the neonatal period, no risk factors associated with the disease were detected. During early life, eczema and a short duration of breast feeding (less than three months), occurred significantly more in diabetic cases than controls.
Conclusion: Eczema and breast feeding for less than three months are risk factors for type 1 diabetes in a southern European population. The type, duration, and mode of treatment for infectious diseases during pregnancy need additional investigation as risk factors for type 1 diabetes.

Type 1 diabetes is a disease which occurs as the result of pancreatic β cell destruction in subjects with genetic susceptibility to the disease and exposure to still undefined environmental factors.[1 2]

Incidence rates for type 1 diabetes within Europe show a north-south gradient, with higher figures in northern than southern latitudes.[3 4] Finland is the country with the highest annual incidence of 40.5 cases per 100 000 people aged <15 years, while in Italy the incidence ranges between 7 (continental Italy) and 37.2 (Sardinia) cases per 100 000 each year in the same age group. In a five year (1989–98) prospective study of the incidence of type 1 diabetes in Rome and the surrounding region (Lazio-Central Italy), within the 0–14 year age group we found an incidence of 8.6 per 100 000 each year (95% CI 7.97 to 9.27), with a higher incidence in the 5–9 year old age group (10.9 per 100 000).[5] More recent data extended to eight years of study has confirmed that in the Lazio region there is a low disease incidence, with a slight increase in the very young age group[6] in line with a similar trend in Europe.[4] Compared to Sardinia where type 1 diabetes is more frequent in males than females, no significant sex differences were observed in type 1 diabetes in the Lazio region.[6]

Environmental factors playing a role in the pathogenesis of type 1 diabetes may differ substantially from population to population. More specifically, disease incidence in one geographical area may differ from another because of different exposures to a given risk factor or because of difference between the populations' genetic susceptibilities to that risk factor. For instance, recent research in Finland, a country with few hours of annual sunlight (1900 hours per year), has focused on lack of vitamin D as a major risk factor.[7] Northern Europe has also reported an increased risk for type 1 diabetes from early life consumption of cows' milk,[2] but an Austrian study, presumably with a different gene pool, failed to confirm this association.[8] In central Italy no data has been available on prenatal or early life environmental risk factors for childhood type 1 diabetes.

A case-control study was therefore designed to identify which environmental factors might contribute to the occurrence of type 1 diabetes in the population of the Lazio region, and to calculate the risk of exposure. A registry of type 1 diabetes cases, established in the Lazio region in 1989 as part of the EURODIAB ACE project,[5] provided us with a unique database of diabetic case participants. A survey was carried out by means of a questionnaire focusing on a number of environmental factors to which cases and controls may have been exposed during gestation, the neonatal period, and early years of life.

SUBJECTS AND METHODS

A population based type 1 diabetes register was established in Lazio region in 1989 and was validated by the standards of EURODIAB ACE which defines type 1 diabetes in accordance with the World Health Organisation (WHO) classification. The type 1 diabetic patients selected for this study (330 cases) were diagnosed within the EURODIAB ACE study, and were born between 1977 and 1989. Population based control subjects born in the same period were chosen from primary and secondary school enrolment records in the region, and the selection process was designed in collaboration with the EURODIAB Substudy 2 Group. It is well known that children within the same school may tend to have more similar characteristics compared to children from different schools. (There is a social class correlation among schools.) Therefore, to minimise the effect of this correlation and ensure an accurate sampling of the general population, we selected our controls from 301 different schools while maintaining the same distribution by type (for example, scientific, classic, technical) and grade (elementary, middle, senior high) as exist in Rome and its province.

Questionnaires were distributed to all parents with affected children and those of control subjects. The parents received questionnaires either from their child's physician for cases or from the child's teacher for controls. The teachers participating in the survey were trained to motivate the parents of the control subjects to fill out the forms accurately and thereby minimise the bias of inaccurate recall frequent in retrospective

studies[9] because of a lack of interest from the control respondents. The teachers were given detailed written information packets prepared according to the same protocol used in the EURODIAB Substudy 2 study, which provided them with instructions on gathering participant information and obtaining consent, preparing interview schedules, and completing the record sheet.[10] Permission was obtained from the ethical committee at the coordinating centre.

Of 330 cases of type 1 diabetes born between 1977 and 1989, we were able to contact 150 patients (45% of the eligible cases) who maintained the same phone number or address or frequented the same diabetes clinic as recorded in the register. The remaining 55% of the patients were not able to be contacted because of change of residency. All 150 contacted patients replied by filling out the questionnaire. Of 1200 questionnaires distributed among controls, 1100 replied (91%)—a surprisingly high percentage, as a result of the motivational preparation of the parents. Based on the replies obtained from cases (n = 150), 750 were randomly taken from controls—that is, matching one case with five controls by age, which is appropriate for this type of analysis.

In the questionnaire, we presented questions related to exposure to risk factors occurring during the mother's pregnancy, the neonatal period, and early years of life. Among such questions were: mother's pregnancy history including drugs taken; occurrence of autoimmune, infectious, and allergic diseases during the neonatal period; birth characteristics and diseases which occurred during the neonatal period; vaccinations received by the subjects; and eating habits during early life, including vitamin D supplementation.

Core variables were transferred from the questionnaire to a standardised record sheet, and the data computerised centrally.

Statistical analysis

For each variable analysed, the odds ratio (OR) and 95% confidence intervals (CI) were calculated (Stat-View). Conditional logistic regression analysis was also performed to adjust the possible confounders. The odds ratio after adjustment is similar to the pooled odds ratio.

RESULTS

Of those who sent back questionnaires, 90–100% responded to all the questions; there was no significant difference between cases and controls in the number of questions answered. Table 1 shows the frequency distribution of age among cases and controls. Overall data analysis showed an expected association with family history for type 1 diabetes significantly increasing the risk of developing the disease (logistic regression analysis: OR 1.17, 95% CI 1.05 to 1.76). Two per cent of patients had a diabetic father, 1.4% had a diabetic mother, and 5.2% had at least one sibling with type 1 diabetes. No patients had both parents or more than one sibling affected by the disease.

Table 1 Frequency distribution of cases and controls by age

Age at time of study (y)	Cases (no.)	Controls (no.)	Total (no.)
6	3	15	18
7	5	25	30
8	13	68	81
9	11	52	63
10	10	50	60
11	17	110	127
12	28	109	137
13	18	96	114
14	14	70	84
15	17	85	102
16	7	35	42
17	6	30	36
18	1	5	6
Sex			
M	76	378	454
F	74	372	446
Total	150	750	900

Table 2 Environmental factors operating during pregnancy

Risk factor	OR	95% CI
Maternal habits		
Smoking	0.85	0.52 to 1.36
Coffee	0.89	0.60 to 1.31
Tea	0.66	0.40 to 1.08
Maternal diseases		
Pregnancy induced hypertension	0.51	0.15 to 1.7
Gestational diabetes	1.35	0.37 to 4.91
Autoimmune diseases	1.16	0.49 to 2.69
Infectious diseases	1.73	1.13 to 2.64
Drugs used during pregnancy		
Antibiotics	0.61	0.13 to 2.69
Analgesics	1.43	0.71 to 2.86
Antihypertensives	0.83	0.09 to 2.31
Antiemetics	1.22	0.64 to 2.31
NSAIDs	0.49	0.06 to 3.84
Antiepileptics	10.00	0.90 to 11.03
Steroids	0.97	0.53 to 1.73
Thyroid hormones	5.02	1.00 to 25.13
Insulin	1.24	0.13 to 11.18

Environmental factors occurring during pregnancy

For environmental factors occurring during the mother's pregnancy (table 2), no significant differences between patients and controls were observed on the use of coffee, tea, and smoking habits. Among maternal diseases occurring during pregnancy, infectious diseases were more frequent in cases than in controls (OR 1.73, 95% CI 1.13 to 2.64) (the questionnaire did not specify between bacterial or viral infections). However, the occurrence rates of gestational diabetes and autoimmune diseases in the mothers did not differ between the two groups, nor did maternal consumption of drugs.

Environmental factors during the neonatal period

Birth characteristics (table 3) did not vary between patients and control subjects: frequency of birth in the hospital (95.0% cases *v* 95.0% controls), full term birth (87.3% cases *v* 84.0% controls), average time of gestation (38.2 weeks for cases *v* 38.2 weeks for controls), average birth weight (3336 g for cases *v* 3356 g for controls), and postpartum treatment (13.3% cases *v* 14.0% controls). Furthermore, caesarean section (OR 1.30, 95% CI 0.87 to 1.94), infections (OR 1.20, 95% CI 0.33 to 4.30), respiratory diseases (OR 0.86, 95% CI 0.29 to 2.52), and

Table 3 Environmental factors during neonatal period

Risk factor	OR	95% CI
Birth in hospital	1.70	0.59 to 4.86
Full term birth	1.44	0.81 to 2.56
Caesarean section	1.30	0.87 to 1.94
Post-birth paediatric treatments	0.94	0.55 to 1.59
Infections	1.20	0.33 to 4.3
Respiratory diseases	0.86	0.29 to 2.52
Jaundice	1.18	0.79 to 1.75

Table 4 Environmental factors during early life

Risk factor	OR	95% CI
Rubella	1.32	0.89 to 1.97
Measles	0.87	0.59 to 1.26
Varicella	0.91	0.63 to 1.33
Pertussis	0.91	0.62 to1.31
Parotitis	1.17	0.82 to1.69
Thyroid disease	18.45	3.79 to 89.82
Rheumatic disease	1.26	0.14 to 11.33
Coeliac disease	0.33	0.01 to 2.55
Eczema	1.96	1.16 to 3.32
Rhinitis/conjunctivitis	1.02	0.64 to 1.62
Asthma	0.9	0.57 to 1.43
Vitamin D supplement	1.22	0.82 to 1.83

Table 5 Breast feeding and weaning in the first year of life

Risk factor	OR	95% CI
Duration of breast feeding <3 months	2.12	1.38 to 3.26
Beginning of weaning <3 months	1.81	1.06 to 3.07
End of breast feeding <3 months	2.03	1.24 to 3.33
Cows' milk before 3 months	1.49	0.72 to 3.11
Meat before 3 months	1.77	0.18 to 17.25
Fish before 3 months	1.33	0.15 to 12.03
Fruit before 3 months	0.40	0.05 to 3.09
Eggs before 3 months	2.20	0.42 to 11.50
Vegetables before 3 months	0.76	0.09 to 6.27

jaundice (OR 1.18, 95% CI 0.79 to 1.75) were not significantly different between cases and controls.

Environmental factors during early life

During early life (table 4) thyroid gland disorders (OR 18.45, 95% CI 3.79 to 89.82) were more frequent in cases than in controls; this did not reach statistical significance because of the wide confidence interval. In contrast, eczema (OR 1.96, 95% CI 1.16 to 3.32) was significantly more frequent in cases than in controls. No significant differences were observed between the two groups for receiving mandatory and non-mandatory vaccinations or in vitamin D supplementation.

Regarding feeding habits during the first year of life, it was discovered that less than three months of breast feeding, early commencement of weaning, and suspension of breast feeding in favour of cows' milk formula before 3 months of life were all higher for type 1 diabetic cases than for controls (table 5). However, because of the confidence levels, the only factor reaching statistical significance as a risk factor for type 1 diabetics was short duration (<3 months) of breast feeding.

Logistic regression analysis for overall risk factor significance

Table 6 presents the logistic regression analysis, adjusted for sex and age, for those risk factors which overall have been found to be statistically associated with type 1 diabetes: family history of type 1 diabetes, presence of infectious diseases during mother's pregnancy, breast feeding <3 months, and occurrence of eczema.

DISCUSSION

This population based study in Rome and its province is the first one describing environmental risk factors for type 1 diabetes in a well defined geographical area of Italy and in a continental Italian population where incidence data for the disease are available.[5] Overall, our results confirm what has been reported in other Caucasian populations, but differ from Austria where intake of cows' milk was not associated with type 1 diabetes.[8]

In conducting our study, every effort was made to trace cases, but we lost contact with 55% of them because the families had moved to different regions. It is possible that moving may have led to potential bias in case responses because of different characteristics of the "movers" such as parental income, lifestyle, and social class. Nevertheless, the number of questionnaires obtained from cases (n = 150) comes from one single region, and is still higher than the 102 cases (from eight different European centres) reported by the EURODIAB Substudy 2 Group.[11]

In this case-control study we analysed several environmental factors that may have occurred during gestation and early life to influence type 1 diabetes development, with the aim of identifying non-genetic risk factors associated with the disease. As expected, family history of type 1 diabetes and infectious diseases during the mother's pregnancy (without distinction between viral or bacterial infections) were associated with type 1 diabetes.[2] These findings are consistent with the concept that autoimmune genes are clustered, and families susceptible to one immunologically mediated disease may also be susceptible to others. Our data regarding breast feeding habits indicate that as in other parts of Europe,[2] in the Lazio region breast feeding for less than three months is a risk factor for type 1 diabetes. Therefore, even in a southern European region such as Italy, breast feeding and avoidance of cows' milk in early life is an important preventative factor for type 1 diabetes.

An observation of interest, especially as compared to other studies of this kind, was the slightly higher incidence of caesarean section births among cases compared to controls. Similar to the recent report of the EURODIAB Substudy 2 Group (not including continental Italy),[10] we found that among prenatal factors, caesarean section delivery was slightly, but not significantly, associated with type 1 diabetes in our population. However, in both the UK and Sweden, caesarean section birth was found to be a significant risk factor for type 1 diabetes.[12 13] They suggest that exposure to anaesthesia given during a caesarean section may have a deleterious effect on β cells in individuals genetically susceptible to type 1 diabetes.[14–16]

Recently great attention has been paid to the role of vitamin D in type 1 diabetes. Vitamin D supplementation has been reported to have a protective effect against type 1 diabetes in

Table 6 Logistic regression analysis for risk factors which have been found to be significant

Risk factor	χ^2	p value	OR	95% CI
Family history of type 1 diabetes	8.092	<0.004	1.17	1.05 to 1.76
Presence of infectious diseases during mother's pregnancy	8.211	<0.004	1.60	1.32 to 2.20
Breast feeding <3 months	5.649	<0.017	1.74	1.40 to 2.45
Occurrence of eczema	4.067	<0.043	1.61	1.25 to 2.66

northeast Europe,[17] and cod liver oil supplementation during pregnancy was reported to be associated with a reduced risk of type 1 diabetes in offspring.[18] These findings suggest that vitamin D or the n-3 fatty acids, eicosapentaenoic acid and docosahexapentoic acid in the cod liver oil, or both, have a protective effect against type 1 diabetes. However, in our study, no significant correlation was found between vitamin D and risk for type 1 diabetes. This result may be partly explained by the fact that the Lazio region is located in a Mediterranean area where a sunny climate (2500 hours of sunlight annually) predominates throughout the year. Ultraviolet irradiation of the skin is a major source of this vitamin, and skin synthesis of vitamin D can obtain levels up to 10 000 IU (250 μg) when the whole body is exposed to the sun.[19] Thus, even without supplementation, the entire population, including those at risk for type 1 diabetes, may have sufficient levels of vitamin D in their system to prevent disease onset.

By our logistic regression analysis, presence of eczema in early life was found to be associated with type 1 diabetes, in contrast to the Eurodiab Substudy 2[11] and Meerwaldt and colleagues,[20] where atopic disease and asthma were associated with significant reduction in risk for type 1 diabetes. Allergic contact dermatitis or eczema is a type IV T cell mediated reaction occurring in sensitive individuals with lesions characterised by a mononuclear infiltrate consisting mainly of T cells.[21] Therefore, we can consider eczema to be an immunologically mediated disease, and it is not surprising that it is associated with type 1 diabetes. Finally, although it did not reach statistical significance, thyroid disorders were more frequent in cases than in controls. Among such disorders, hypothyroxinemia, which is common in the preterm infant, is associated with a number of other autoimmune conditions as recently reported.[22]

Our data for the association of eczema with type 1 diabetes and the lack of association of coeliac disease with type 1 diabetes differ from that of other studies in the literature. Such variance reinforces the idea that the same environmental risk factor may have a different impact on different Caucasian populations and further shows the complicated "puzzle" of the role of the environment in the pathogenesis of this disease.

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Authors' affiliations

N Visalli, S Manfrini, P Pozzilli, Unit of Diabetes and Endocrinology, Interdisciplinary Centre for Biomedical Research, University Campus Bio-Medico

L Sebastiani, E Adorisio, A Conte, A L De Cicco, R D'Elia, Institute of Public Health, University of Rome "La Sapienza"

IMDIAB GROUP: Paolo Pozzilli, Natalia Visalli, Silvia Manfrini, Elvira Fioriti, Giusy Coppolino, Luciana Valente, Chiara Guglielmi, Giuseppina Beretta Anguissola, Flavia Costanza, Anna Lisa Montemari, Maria C Matteoli, Patrizia Patera, Antonio Crinò, Stefania Corbi, Sabrina Spera, Concetta Suraci, Marco Cervoni, Anna Cantagallo, Giancarlo De Mattia, Maria R Cassone Faldetta, Maria L Manca Bitti, Giovanni Marietti, Federica Ferrazzoli, Carla Bizzarri, Dario Pitocco, Giovanni Ghirlanda

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